precisely the same way with the spark spectrum as with the flame spectrum, and that the red cadmium line behaves in the same way as the others. The magnetic effect is better seen, from a direction perpendicular to the line of force, when a nicol is interposed in the path of the light, but rotation of the nicol through 90° cuts it entirely off, accurately so when a small spark is the source of light.—May 31.]

Fifth Report to the Royal Society Water Research Committee.

By H. Marshall Ward, Sc.D., F.R.S., F.L.S., Professor of Botany in the University of Cambridge. Presented to the President and Council, December 10, 1896.

(Abstract.)

The following is a short *résumé* of the principal points resulting from three years' study of the Bacterial Flora of the Thames.

All the forms have been grown on gelatine, agar, and potato, as well as in broth, milk, and sugar-solutions, and some of them in special media in addition. Moreover, most of them have been cultivated in hanging drops under the microscope, sometimes isolated and under high powers, for long periods, and the course of development of the colonies traced in detail in several cases, and even when this could not be successfully carried to a conclusion, information as to the changes and growth of the organisms has been obtained, which helps to throw light on their behaviour and relationships.

This work has occupied a long time, and these water bacteria do little beyond growing and dividing, so that in many respects this part of the work only starts problems for the future. In some cases, however, the morphological changes observed are helpful in explaining the macroscopic appearances of the colonies, and in any case it is clear that no examination of a bacterium can be considered complete until its life-history has been traced under the microscope.

The number of forms isolated and cultivated amount to eighty, not counting the large number which were either rejected at an early stage as certainly duplicates, or lost during cultivation.

Of these eighty forms some have been distinctly recognised as well known types, *e.g.*, *Bacillus membranaceus amethystinus* (Eis.), *B. fluorescens liquefaciens* (Fl.), *B. fluorescens non-liquefaciens* (Fl.), *B. coli commune*, *Proteus vulgaris* (Haus.), *Bacillus arborescens* (Fr.), *B. prodigiosus* (Ehr), *B. termo* (Cohn), *B. subtilis*, and *Sarcina lutea*.

Others have been referred with less certainty to less well known forms, such as *Bacterium ureæ* (Jaksch), *B. fulvus* (Zimm.), *B. aureus* (Adam.), *Ascococcus* (Cohn), *Micrococcus carneus* (Zimm.), and *M. candicans* (Fl.).

The remainder are either new, or only doubtfully identified as already described forms, or evidently varieties of some of the foregoing.

During the progress of the cultures, large numbers of coloured drawings were made, with the intention of affording means of identification, but it was found that variations are so numerous and so large, that some study of these variations had to be undertaken.

This led to an investigation of the growth of the colonies in gelatine and other media, and an attempt to explain why, and how far, the colonies vary in culture. This necessitated a careful examination of the factors concerned in the development of the shapes, markings, movements, and so forth of the colonies themselves, and to a classification of the characters furnished by these colonies.

One outcome of the above studies was the conviction that two sets of factors are at work in causing the variations found in the colonies. First, the slight variations in the food-materials, temperature, moisture, &c., which cannot be avoided, however carefully the work is done; and, second, variations in the bacterium cell itself as it comes from the river, owing to the exigencies it has been subjected to during its sojourn there. The water of the river is, in fact, a very dilute and indefinite food solution, and just as changes occur when we remove a bacterium from broth to milk or to gelatine, so do such result when we transfer from the river to these media, and the changes induced in all cases depend on how long the bacterium has been in the one medium or the other, as well as upon other factors.

The river water is a very poor food medium, and so we cannot be surprised that in many cases the recently isolated bacteria behave as weakened forms; the recognition of these enfeebled varieties suggests explanations of many of the bad "species" in the literature of water bacteria. My work goes to show, not that species cannot be made out, but that the limits of the species are, in most cases, far wider than is assumed in descriptions—in other words, that many so-called species in the books are merely varietal forms, whose characters, as given, are not constant, but depend on treatment. How far this is true for any given case will have to be tested on the particular form in question.

Very slight variations in rapidity of growth of the individual bacterium, its power of liquefaction, pigment production, capacity of fermenting, and so on, lead to comparatively very great differences in the appearance of the colonies formed in a given time on, or in, a medium like gelatine, the composition, aëration, hygroscopicity,

density, &c., of which is changing all the time, and since every variation in temperature, light, air-supply, &c., affects the matter also, I find abundant causes for the variations which otherwise appear so astonishing. It results, therefore, that the attempt to determine species of bacteria by ordinary macroscopic methods leads to difficulties of the same kind as would be met if we tried to differentiate species from the marks presented by masses of trees in forests from a distance—say in a balloon. A forest of a given species of tree would appear very different at different seasons, and according to its age, the kind of soil, climate, and so on, and the treatment it had received previous to planting.

In order to emphasise my own impressions of the specific value of the forms isolated, and at the same time leave it clear what are the differences between the various forms or varieties themselves, I have grouped them into types. Each group contains a type which I regard as probably a species, of which the other forms included are varieties. These varieties are fully described and figured, and would certainly be regarded as species in most books consulted. The reasons for grouping them as I have done are fully discussed with the literature under each. Here, of course, I can only give a very brief summary of the groups as follows :—

Group I is made to contain a form which appears to be identical with the *Bacterium ureæ* of Jaksch, and forming curiously characteristic dull stearine-like plate colonies. It was not uncommon in the river, but the want of salient features rendered it comparatively uninteresting, and no prolonged study of other varieties of it was undertaken.

Group II was constituted for a type which in certain cultures developes an intense violet colour exactly like that of a strong solution of gentian violet. It was not common—at any rate in the typical form—but it was easily cultivated, and two varieties were isolated and kept under observation for many months.

The type accords best with Jolles and Eisenberg's *B. membranaceus amethystinus*, and forms the deep blue violet zoogloea-membrane described for that species, but three years' study of the variations in culture convinces me that several violet bacilli described in the literature are identical with this, or at least cannot be separated by the characters given.

A marked tendency to lose its power of forming pigment, and to grow as a pure white form, characterises this bacillus. It is the species with which I obtained the bacterial photographs exhibited in 1894, and is very sensitive to light. The spectroscopic characters of the pigment were determined, and the bacillus itself cultivated under high powers.

Group III comprises the common *B. fluorescens liquefaciens*, very

abundant in the river. Four varieties were isolated and cultivated. It is the same as that previously described by P. Frankland as *B. viscosus*, and by Schmolck as *B. fluorescens nivalis*. Some of the varieties liquefied very feebly or not at all, suggesting that *B. fluorescens non-liquefaciens* may be only a form of the present species, a conclusion borne out by comparing the two side by side, on, and in, various media.

Group IV comprises the non-liquefying forms of the type *B. fluorescens non-liquefaciens*, extremely common in the river, and of which eight were isolated and kept in culture.

These eight varieties differ in details, but the differences are not constant, and their behaviour suggests the identity of several so-called species of green fluorescing bacteria, hitherto regarded as distinct, *e.g.*, the Bacillus of Lesage, that of Adametz, Lustig's *B. aquatilis fluorescens*, Flugge's *B. fluorescens putidus*, and certain forms described by Frick and Zimmermann.

The fact that some of these varieties were pathogenic and some not, points to the possibility of there being two species, but as experience shows that pathogenicity varies as well as other characters, this could not be relied on, any more than the differences in size, colour, &c.

Group V embraces a large series of forms comprising the typical *B. coli communis* and a number of varietal and allied forms. In all sixteen varieties were studied in detail, some of them very closely and for long periods. They are common in the river, especially in summer, though some of the forms show variations so marked that few observers would be likely to suspect their close relationships. For instance, varieties occurred incapable of coagulating milk; others which formed no gas-bubbles in gelatine; others not pathogenic for guinea-pigs. Some other forms, suspiciously like *B. coli* in other respects, but forming capsules, or liquefying gelatine to a greater or less extent, are placed in different groups in deference to usually received opinion, though I am convinced of their close relationship.

High-power cultures show considerable differences in size of rodlets, and facts were obtained of value in understanding the very great differences observed in the macroscopic characters.

Group VI comprises a series of forms centering around the type of Hauser's *Proteus vulgaris*, and of which eleven forms were isolated and cultivated.

One of the most striking features of this type is its variability as to liquefying power; another is the concurrent variations in motile power of the plate-colonies. I recognised the types named *P. mirabilis* and *P. proteus* by Hauser, and during cultures extending over three years have convinced myself that not only are these and his *P. Zenkeri* merely varieties of the same form differing in liquefying

power, but that they vary in several directions towards powerfully liquefying forms of the *B. termo* type, on the one hand, and yellow chromogenic forms, like Zimmermann's *B. ochraceus* and P. Frankland's *B. arborescens*, on the other.

A careful and prolonged study of the plate-colonies and "wandering islets" was made under high powers, throwing light not only on the movements of these colonies over the surface of the gelatine, but also on the structure of all such colonies as are composed of tresses, zoogloea-clumps, and contoured groups of filaments, motile or not.

Drawings were made illustrating the movements and changes in form of the "wandering islets" at intervals, and showing the individual movements of the bacilli on which these depend. Evidence is also given showing that the differences in size, vigour of movement, and liquefaction, and chromogenic power, depend on the vigour of the organism as it comes from the river, as well as on variations in conditions of culture.

Group VII includes seven forms evidently closely allied to the last group, if really distinct. They are remarkable for their more or less pronounced yellow pigment formation, and are termed the yellow Proteus type.

I have identified some of the varieties here collected together as Zimmermann's *B. radiatus* and *B. ochraceus* and P. Frankland's *B. arborescens*, and there can be little doubt that other yellow chromogenic forms described will have to be included. Among these, a number of "non-liquefying" yellow forms may also have to be counted when they have been sufficiently studied in detail.

Group VIII includes a form which varied so much that I had to make numerous separation cultures at various periods during the two years it was kept in culture, to satisfy myself there was no admixture. Its most characteristic features were the star-like character of the plate-colonies and the presence of a bluish iridescence when held up, but a yellow pigment was also formed in varying degrees of intensity. In other characters it comes near Group IX, on the one hand, and the yellow Proteus type of Group VII on the other, but its peculiarities were so puzzling that I kept it apart.

Group IX comprises a series of golden-yellow liquefying forms, of which five varieties were studied. The type is that of Zimmermann's *B. fulvus* and *B. subflavus*, and, while they differ in liquefying power, all form deep chrome-yellow pigment, like yolk of egg. The group is clearly allied to the yellow Proteus group, and seems to connect P. Frankland's *B. aquatilis* and the true Proteus type with *B. arborescens* and *B. ochraceus*, the differences depending largely on the formation of zoogloea or not.

Group X consists of a series of very common non-liquefying forms, of which three varieties were studied in detail. The type appears

nearest to Adametz's *B. aurcus* and P. Frankland's *B. aurantiacus*. They grow very slowly, and seem to be feeble forms. From their behaviour during culture and comparison with the last group, I am compelled to regard these non-liquefying yellow forms as merely weakened varieties of the last group; this is borne out by the tendency to liquefaction shown by some of them after being cultivated for some time, as well as by the enfeeblement of liquefying power shown by some of the older cultures of the yellow Proteus group. It is probable that they are simply species of the Proteus group, so weakened by their prolonged sojourn in the river that they can only be cultivated with difficulty in the new environment of the gelatine media, &c. This being so, the whole of the series included in Groups VI to X inclusive may be varieties of one or two species, since I find that, great as the differences seem between the extreme forms, the varieties studied present characters passing imperceptibly into one another. This result is also in accordance with the fact that so many yellow varieties, impossible to separate satisfactorily, have been isolated from water and described by other observers.

Group XI is made to include a colourless capsuled form, not very common in the river, and reminding one of Cohn's *Ascococcus*. Two sets of cultures of it were carried out in detail, and a very characteristic feature was discovered in the plate-colonies, viz., the presence of curious vermiform zoogloea masses imbedded in the colonies; these are not intruded forms, but merely growth-phases of the same species. So far as can be judged, the "species" seems new.

Group XII comprises a series of yellow capsuled bacilli, not uncommon in the river, and morphologically conforming to the type of Friedländer's bacillus. I cultivated five varieties of it. The most characteristic feature is the formation of extremely tough zoogloea-colonies, often looking like the roe of a fish. There is some variation in the degree of liquefying power, and much difference in consistency and other characters, but the sum of all the diagnoses leaves no doubt that all the forms belong to one species.

Group XIII comprises a series of forms which produce a crimson pigment. The type is that of *B. prodigiosus*, and five varieties were isolated and very closely investigated. Colourless varieties were developed in cultivation, and the pigment again restored by suitable treatment. Weakened forms, and considerable differences in liquefying power were also found. Koch's *B. indicus*, Breunig's *Kieler bacillus*, Frank's *B. ruber*, Dowdeswell's *B. rosaceum metalloides*, and Zimmermann's *B. miniaceus* are all identical with this form, or, at least, can be matched by the varieties arising in culture.

Group XIV comprises a series of rapidly liquefying, colourless forms, very common in the river at all times, and conforming to the type of *B. termo* as amended by Macé. I made a prolonged study of

five varieties, and found considerable differences as regards rapidity of liquefaction, anaërobism, and other characters. The series includes Eisenberg's *B. liquefaciens*, Frankland's *B. liquidus*, and Zimmermann's *B. punctatus* and *B. devorans*, and brings together a large series of incompletely described forms with more or less probability. The type is one of the commonest in the Thames, and a pronounced putrefactive bacterium.

Group XV includes three varieties not uncommon in the river in the summer, and conforming to the type of *B. subtilis* in general behaviour and in the characters of their highly resistant spores, the germination of which was carefully traced. The total behaviour of these varieties points to confusion between the hay bacillus (*B. subtilis*) and the potato bacillus (*B. mesentericus*), and I am driven to the conclusion that these two "species" are either identical or more similar than is usually assumed. In any case both these forms are matched by the varieties in this group, which my examination suggests are varieties of one species. The wrinkled growth on potato cannot be relied upon to separate them.

Group XVI comprises a series of varieties of a yellow *Sarcina* of the type *S. lutea*. I obtained five varieties, showing considerable differences in liquefying power, and it was interesting to find that the *Sarcina* form—"packet form"—is not always maintained. My cultures unite Frankland's *S. liquefaciens*, Lindner's *S. flava*, and Schroeter's *S. lutea* as mere varieties of one and the same species.

Group XVII was made for a rose-pink or cerise-coloured micrococcus of the type of Zimmermann's *M. carneus*, not common, but isolated several times in the winter of 1894—1895. The most interesting point was the discovery that in early stages of division, followed under high powers, it develops as a *Sarcina*, and several facts point to the conclusion that the *Sarcina* form is a mere result of slow development—*e.g.*, in acid media—of *Staphylococcus* forms of *Micrococcus*. My cultures unite *M. carneus* with Maschek's *Coccus ruber*, Flügge's *Micrococcus cinnabareus*, Zimmermann's *M. cinnabarinus*, and certain forms often termed *M. roseus*, as well as Schröter's *Sarcina rosea*, as mere varieties of one form.

Group XVIII is made for a curious form which serial cultures under the high power shows to be no schizomycete at all, but a truly branching micro-organism with acropetal growth and other characters which place it in the category of true fungi. Nevertheless it breaks up into minute coccus-like oidial joints, so similar to a short bacterium or oval coccus that it would inevitably be taken for such if examined by ordinary methods only. I referred to it in 1895 as a "false bacterium," and its history affords an excellent proof of the necessity of microscopic cultures in these investigations. It presents

undoubted resemblances to Löffler's *B. diphtheriæ*. It was not found to be pathogenic to guinea-pigs by Dr. Kanthack.

Group XIX was made to contain a white micrococcus of the type *M. candicans* of Flügge. It is very common in the river, and was kept under observation a long time, with interesting results; for although typically a non-liquefying form, it becomes capable of liquefying gelatine in time, and the plate-colonies and other cultures show variations which lead me to connect *M. candicans* with Zimmermann's *M. concentricus*, Tataroff's "schminkeweisser *Streptococcus*," and some other forms, as mere varieties of one and the same species.

Group XX contains a form only isolated once from the Thames, but studied very thoroughly in 1894. It develops lemon-yellow liquefying colonies on gelatine, and presents close resemblances to *Sarcina lutea*, but it is a fairly large bacillus, and no attempt to convert is into a *Sarcina* form succeeded. All efforts to match this form among those described have failed, though incompletely examined forms by Maschek, Unna and Tommasoli, and Adametz present resemblances, so far as their imperfect diagnoses go. It may be necessary to name it as a new species.

Group XXI contains two varieties of a short oval form, or coccus, principally characterised by the development of a red pigment, varying from pale salmon-pink to deep rosy-scarlet, but also differing in details of shape and adaptation to temperature, and a few other points. Nevertheless it was difficult to give the varieties specific rank on the evidence. I have been unable to identify this form.

Group XXII comprises two forms, not completely investigated, and nearer the type of Group XIV than any others of my groups. Both seem common in the river.

The question of the pathogenicity or otherwise of these forms was naturally raised, and was kindly tested for me in several cases by Dr. Lazarus Barlow, and at much greater length by Dr. Kanthack, through whose hands nearly all the forms have passed. In the following summary pathogenic means pathogenic to guinea-pigs. Pathogenic varieties occurred in Groups IV, V, VI, VIII, XI, XIII, XIV, and XVII, with considerable variation in detail, while the other groups seem to be harmless. The most markedly pathogenic forms were in Group V (type of *B. coli commune*). Those in Group IV aroused the suspicion that *B. pyocyaneus* comes here; and it is interesting to note that *B. prodigiosus* (Group XIII) was pathogenic in some cases.

Of course no full test of pathogenicity is afforded by the few trials made in this way, but the positive results obtained at least show that a not inconsiderable number of the river bacteria are pathogenic, or can become so in culture. Not less important is the

fact that pathogenicity, like the other characters, is variable, as is abundantly shown by Dr. Kanthack's experiments.

In conclusion, the results show that—

1. Very many forms occur in the Thames, some of which are pathogenic under certain conditions.

2. The "species" of the descriptive hand-books—principally medical—are frequently not species at all, in the botanical sense, but varieties, or growth-forms, the distinctive characters of which are not constant. These so-called species need revision and grouping around types, which may turn out to be the true species.

3. The characters derived from the behaviour of colonies are not sufficient for the determination of species, and how far they may be employed in conjunction with other characters will only be elucidated by advances in our knowledge of the way the colonies are built up by the growing bacteria on the given media.

4. The effects of definite changes in the environment on the media are of great importance, but have hardly been noticed as yet. Plate-colonies on gelatine, for instance, develop quite differently, according to the condition of the gelatine; so that a feeble and slow-growing bacterium produces colonies quite unlike those developed by the same species when vigorous and quickly growing, not only owing to its peculiarities of growth as a feeble form, but also because the gelatine has altered during the intervening period.

5. The effect of changes of the environment on the growing organism itself is recognised as important.

6. With especial reference to the Thames bacteria, the past history of the organism isolated from the river implies causes of variation. The river water is a poor nutritive medium, and the organism is exposed to great changes of temperature, light, movement, &c., during its sojourn therein. Consequently the time it has been in the river affects the behaviour of the organism when isolated, just as we know that a bacterium is affected by the previous conditions of its culture in other media. Hence two colonies on a plate may look very different, and yet belong to the same species, one being developed from a cell that had been many days or weeks in the water, the other from one that had only been there a few hours. It may need weeks or months of cultivation under constant conditions to establish the identity of the two.